EXPERIMENTAL EPIPHYSIAL CARTILAGE GROWTH ANOMALIES

E. STOREY*, MELBOURNE, AUSTRALIA

From the Department of Pathology, University of Melbourne

Endochondral bone growth is a complex and highly integrated process which may be affected by a large number of mechanical, hormonal and chemical factors. In the growing long bone the cartilage provides a model for subsequent ossification and a means of interstitial growth under mechanical loads of different intensity, duration and directions. In man at maturation the epiphysial cartilage is replaced by bone and longitudinal growth ceases. However, in a group of conditions of diverse, questionable or unknown etiology, cartilage remnants, presumably derived from the epiphysial plate, are found in the epiphysis, metaphysis or shaft. Such aberrant cartilage is present in long-standing rickets; in the cartilage dystrophies such as enchondromata or eechondromata; metaphysial dysostoses; and osteochondroses such as Legg-Perthes' disease.

In these conditions the mechanism whereby aberrant cartilage becomes included or develops in bone is not known. With the exception of rickets, few have been induced in experimental animals, and the nature of the cartilage changes is still subject to speculation. Recent studies designed primarily to explore the effect of large doses of stable strontium on bone have demonstrated remarkable changes occurring in the epiphysial plate. Although the effects of stable strontium have no immediate relation to observed conditions in man, the nature of the cartilage change may provide a clue to some aspects of the development of clinical conditions and, furthermore, a means whereby the basic mechanisms of the cartilage plate deformity can be investigated in more detail.

The nature of bone and chemical changes occurring during strontium rickets has been described before (Shipley, Park, McCollum, Simmonds and Kinney 1922; Storey 1961, 1962). Although early changes are similar to those seen in avitaminosis D rickets in rats, later ones are different. Hypertrophic cartilage may be left behind in the developing bone in the early stages, but as the animals grow, other forms of cartilage abnormality appear. In general terms two patterns of change in the epiphysial plate, not hitherto described in detail, are shown by histological study of undecalcified bone sections: 1) localised failure of endochondral ossification followed by formation of multiple nodules of cartilage sequestrated into the developing epiphysis or metaphysis; and 2) disorganisation of the cartilage plate with alteration in form of the epiphysis.

MATERIAL AND METHODS

In these experiments stable strontium in the form of SrCO₃ was fed to rats at a level of 2-6 per cent in the diet, the animals being killed at intervals during the next twelve months. Both decalcified and undecalcified sections (Ueckert 1960) were prepared and stained with Ehrlich's haematoxylin and eosin, periodic acid-Schiff reagent, tartrazine, Von Kossa's stain for calcium and toluidine blue. Details of experimental procedures have already been published (Storey 1961).

RESULTS

General changes occurring during strontium administration were reported by Storey in 1962. Three aspects of these can now be recorded in detail.

Localised failure of endochondral ossification—In young rats given strontium diet initially the epiphysial cartilage plate widens but, later, irregular calcification occurs and the width of the plate is reduced to nearly normal. At this stage localised defects in endochondral ossification occur, usually in the centre, but occasionally at the periphery of the plate. In older animals

* Now Professor of Conservative Dentistry, University of Melbourne.
the initial stage of cartilage widening may not occur and the first defect, seen after three or four months on the diet, may be accumulation of osteoid in the middle part of the metaphysis. With the staining methods used, the sequence of events is as follows. At the hypertrophic zone of cartilage, where matrix calcification normally occurs, a small area a few cells wide fails to calcify. Instead of regular invasion of vessels along cartilage columns, both cells and matrix are removed. At this site neither Von Kossa, PAS nor haematoxylin stains reveal calcification of matrix, yet removal of tissue occurs. Vessels penetrate the area and small or even large multinucleated giant cells are present at uncalkified cartilage margins (Fig. 1). At the same time osteoblasts lay down uncalkified osteoid with a coarse irregular woven structure in the metaphysis. This is most apparent because calcified matrix is absent, in contrast to adjoining areas where it is present in longitudinally arranged metaphyseal trabeculae.

With further development of the defect the osteoid tissue assumes first a wedge shape, with the base next to the epiphyseal cartilage and the apex pointing toward the marrow cavity. Later, it may be rectangular (Fig. 2). In some animals more normal endochondral ossification reoccurs and osteoid wedges are found some distance from the cartilage plate, separated from it by longitudinally arranged trabeculae. The osteoblasts at the osteoid margins become smaller and deeply stained with haematoxylin; vessels are no longer visible and fatty marrow fills the intertrabecular spaces of the osteoid wedges. Although occasional patches of calcification occur in osteoid even after twelve months on strontium diet, most of the osteoid wedge formed earlier remains uncalkified in the marrow cavity.

Sequestration of cartilage nodules—Following the localised failure of endochondral ossification, folds or invaginations may occur in the cartilage plate. These usually are at the margins of osteoid wedges and involve all layers of the epiphyseal cartilage; the folds are largely directed into the metaphysis, but sometimes into the epiphysis. As the defect progresses, the proliferative layer of the cartilage lies in the middle of the nodule with irregularly arranged columns of cells radiating peripherally (Fig. 3). After this the continued removal and replacement of cartilage matrix on its epiphyseal aspect leaves the nodule no longer connected to the epiphyseal plate but sequestrated into the metaphysis, where it is either partly or completely surrounded by osteoid trabeculae (Figs. 4 and 5). Such nodules may be single or multiple, and in some cases several appear to have formed successively from the same area of the epiphyseal plate. Others appear at the margins of the cartilage plate and remain within the forming bone shaft.

Single and multiple nodules occur in areas where they must have developed months before death. They appear similar in shape and size to those formed later. Detailed study shows that remodelling is still continuing; vessels are arranged at right angles to the cartilage columns and removal and replacement of cartilage matrix still occurs (Fig. 6). In some areas, particularly in the bone shaft, large nodules of cartilage of different structure occasionally occur. These have small cells and large amounts of intercellular matrix. Intermediate types of structure have not been found.

In some bones cartilage nodules appear in the epiphysis, the same sequence of events occurring as seen in the metaphysis.

Hiatus in the epiphyseal plate—In some animals the cartilage plate becomes discontinuous and fragmented. Usually only one defect, often referred to as a "perforation," occurs and both large and small fragments of cartilage appear in the epiphysis and metaphysis. Along with these changes the epiphysis becomes grossly abnormal in structure and in some cases the articular head of the bone flattens. Fragmentation of the epiphyseal plate may occur either in grossly affected bones, with severe distortion of shape, or in others where the external form remains relatively normal.

The cellular changes are most complex and many variations occur in the deformed cartilage plate. Among these are: 1) Growth of cells of the proliferative layer of cartilage through the subepiphyseal bone plate into the epiphysis (Fig. 7). This may occur over a small
Figure 1—Photomicrograph of epiphysial cartilage. The normal sequence of endochondral ossification has been disturbed and osteoclasts are present at the uncalcified cartilage matrix margins. (Ehrlich’s haematoxylin and eosin, ×500.) Figure 2—Photomicrograph of the upper end of the tibia. An epiphysial invagination is present in the large wedge of osteoid tissue in the metaphysial region. (Ehrlich’s haematoxylin, ×8.)

Photomicrograph of epiphysial cartilage showing a cartilage nodule in the metaphysis with irregularly radiating cartilage cell columns. (Periodic acid-Schiff, tartrazine, ×60.)
Figure 4—Photograph of the lower end of the femur showing cartilage nodules in the metaphysis. (×6.) Figure 5—Photomicrograph of the middle third of the shaft of the tibia showing multiple cartilage nodules in the marrow cavity partly invested by osteoid tissue. (Periodic acid-Schiff, tartrazine, ×20.)

Photomicrograph of part of a cartilage nodule from the same specimen shown in Figure 5. This shows the presence of small vessels and osteoclasts between cartilage cells and adjoining osteoid tissue. (Ehrlich's haematoxylin and eosin, ×500.)
area or a considerable amount of the cartilage plate may be involved. In this way, with columns of cells growing into the metaphysis as well as the epiphysis, large nodules appear within the cartilage plate. 2) Fragmentation of the cartilage plate adjoining the area where a large nodule appears in the metaphysis. The free ends of the cartilage plate fold back upon themselves into the epiphysis to form large nodules or become further fragmented to form a series of smaller cartilage clumps. In the epiphysis such fragments of the plate may lie next to the inner aspect of the articular cartilage where, despite its abnormal site, endochondral growth continues (Fig. 8). In some bones large nodules are found some distance from the epiphysial plate and may either be quite separate or still retain some bony connection with the peripheral remnants of the cartilage. This is well shown in Figure 9 where a large nodule representing the centre of the epiphysial plate of a fibula is some distance from, but still connected by a stalk of bone, to the epiphysial plate.

**DISCUSSION**

The importance of this experimental study is the production of disturbances of endochondral ossification giving various deformities of the epiphysial plate and cartilage nodules in the metaphysial region. Such changes observed in man have been in general single observations of an end result (although serial radiological studies provide a time relation of value). Histological confirmation, however, is lacking, unlike this experimental series. It is to be appreciated that in describing phenomena the use of terms such as "perforation," "rupture," or "invagination" of the epiphysial plate is purely descriptive and does not imply rapid mechanical change.

The general nature of the bone changes occurring during the development of "strontium rickets" has been described before (Storey 1961, 1962). Although early changes are typical of avitaminosis D rickets in rats, later changes at endochondral growth centres are not characteristic. After initial inhibition of calcification and widening of the cartilage plate.
Photomicrograph of part of the epiphysis of a tibia showing a large fragment of the epiphysial cartilage next to the inner aspect of the articular cartilage. (Periodic acid-Schiff, tartrazine, ×40.)

Photomicrograph of the upper end of the fibula showing a large nodule arising from the centre of the epiphysial plate which is some distance down the metaphysis. (Periodic acid-Schiff, tartrazine, ×40.)
mineralisation and endochondral ossification are periodically interrupted with the formation of transverse bands of osteoid trabeculae in the metaphysis. After this a localised failure of calcification and endochondral ossification occurs in the cartilage plate, with formation of osteoid wedges in the metaphysis. This is followed by invagination of the epiphysial plate and the formation and sequestration of cartilage nodules into the metaphysis; in severely affected animals this process may extend until the epiphysial plate "ruptures" and large masses of cartilage appear in the epiphysis and metaphysis. Both nodule formation and rupture of the epiphysial plate are associated, first, with localised failure of calcification and osteoid wedge formation which may vary in site and extent to be single or multiple and, second, with continued growth of the remainder of the epiphysial cartilage; without elongation of the bone, osteoid wedges could not form, nor could subsequent sequestration of nodules occur into the metaphysis.

Development of localised endochondral defects in strontium rickets may be due to many factors. For example, local peculiarities in the vascular distribution and blood flow in different areas of bone and alteration in mechanical stress or change in rate of cellular activity may well determine the precise site and extent of failure of calcification.

The shape of osteoid wedges and their progressive increase in size and well defined margins suggests a definite localisation of the existing structures and indicates that vascular processes are significant in their development. The blood vessels, since they carry the essential nutrient as well as hormones, vitamins and other specific factors, play an important part in the processes involved, but they do not necessarily constitute an etiological feature of the condition. Although vascular architecture may determine the site of inhibition of calcification in strontium rickets it is unlikely to be the sole explanation, for with complete interruption of the metaphysial blood supply to a part of the epiphysial plate, although cartilage cells mature normally the hypertrophic zone fails to calcify; penetration of vessels ceases and cartilage, not osteoid tissue, accumulates in the form of a wedge in the metaphysis (Trueta and Amato 1960). This process is unlike that seen in strontium rickets where, although orderly penetration of vessels along cartilage columns ceases, cartilage material is removed and osteoid trabeculae are formed. This resorption of matrix is associated, not only with increased vascularity, but with numerous multinucleated giant cells adjoining uncalked cartilage margins. This is in contrast to the usual picture of bone and cartilage resorption where only calcified tissue is removed (Storey 1960), although Follis (1952) has seen resorption of uncalked matrix in long-standing rickets in man. These observations are consistent with Trueta and Amato's suggestion (1960) that failure of resorption of hypertrophic cartilage is associated with diminished vascular penetration along cartilage cell columns but that this is not due to failure of calcification of matrix (Trueta 1962).

Another factor which could influence the site of localised endochondral defects is increase in mechanical stress. In the normal animal regional differences in the epiphysial plate are probably related to specific mechanical stresses imposed on the bone (Smith 1962 a and b). Bones in strontium-treated animals are softer than normal, and increased stress could occur along the epiphysial plate and would be expected to influence ossification. However, the experiments of Trueta and Trias (1961) show that when the epiphysial plate is subjected to mechanical compression, ingrowth of vessels along cartilage columns and mineralisation of matrix is inhibited, with subsequent widening of the cartilage plate. Here the histological picture is unlike that seen in strontium rickets and, instead, resembles that seen after interruption of metaphysial blood supply.

A further factor may be local variation in blood flow and permeability of metaphysial vessels. Trueta (1959) has demonstrated that the arterioles empty into large venous sinusoids in the metaphysis and clinical observations suggest that some bacteria may lodge there and be responsible for acute osteomyelitis. This type of process could explain the development of localised defects of calcification, as in vitro studies have demonstrated that the degree of
inhibition of mineralisation of bone matrix in the presence of stable strontium is influenced by the cellular activity of tissue (Lengemann 1960).

After the development of localised calcification defects cartilage nodules appear in the metaphysis. They usually arise close to the junction of an osteoid wedge and more normal metaphysial trabeculae. Here the epiphysial plate invaginates into the metaphysis so that the resultant nodule consists of a core of proliferative cells radiating peripherally in the form of irregular cartilage columns. These nodules maintain their form for long periods of time, unlike transplants of epiphysial cartilage, which can readily become replaced by bone (Schneider 1956). The persistence of nodules in the medullary cavity of long bones is partly related to the presence of the proliferative layer of cells as, where this is included in transplanted cartilage (Schneider 1956), growth continues for a short period. In contrast to this, growth does not occur where cartilage from the maturation or hypertrophic zone is retained in the metaphysis; instead, matrix becomes calcified and eventually replaced with bone during remodelling (Dodds and Cameron 1939, Brashear 1959). Histological study demonstrates that, with continued strontium administration, nodules remain a similar size due to continual growth and transformation to osteoid. However, with withdrawal of strontium from the diet, they calcify and are slowly replaced by bone.

In addition to small cartilage nodules large ones form in association with defects in the epiphysial plate, causing fragmentation. They appear to form in two ways: 1) a large area of the epiphysial plate grows into the epiphysis as well as the metaphysis. With increase in length of the bone, the central cartilage mass with its adherent osteoid wedge remains in the metaphysis; 2) as the epiphysial plate “ruptures” the free ends of the cartilage, next to the perforation, “curl up” into the epiphysis and then may become detached to form one or more cartilage masses. Gross perforations of the plate then become restored, not with cartilage but with bone trabeculae. This sequence of events is consistent with other experimental studies on cartilage plate mechanics as, although regeneration of the epiphysial plate occurs in young animals (Seyle 1934), this is not the rule. Indeed, cartilage repair occurs so slowly that the defect is usually filled with other forms of connective tissue (Cameron 1952).

In older animals, while complete removal of the epiphysial plate inhibits further longitudinal growth, partial resection does not always do so (Banks and Compere 1941; Campbell, Grisolia and Zanconato 1959). Also replacement of cartilage occurs by formation of bone trabeculae (Ring 1955, Ford and Key 1956). Apparently the degree of bony union between the epiphysis and metaphysis determines the extent and nature of growth inhibition (Ford and Key 1956, Friedenberg 1957, Campbell et al. 1959). Similarly in strontium rickets, despite local disappearance and gross deformity of the cartilage plate, continued growth is shown by cartilage masses in the metaphysis some distance from the epiphysial plate. It has been suggested that surgical trauma is responsible for the bony union (Friedenberg 1957) but in the present study in which bones were not subject to any operative procedures, bone trabeculae still filled the epiphysial plate defect. For growth to occur, despite bony continuity of epiphysis and metaphysis, longitudinal pressure exerted by the growing cartilage must be sufficient to induce a rapid rate of bone remodelling in the area. This is shown in the present study where bone trabeculae are orientated in a longitudinal direction along lines of growth both in repair of defects in the tibia and in the fibula. In the fibula the longitudinal growth which occurs could be due, not to the remnants of the epiphysial plate of the fibula, but to the distracting force of the tibia exerted on the adjoining bone. However, when bony union between the epiphysis and metaphysis is extensive the bone remodelling rate is slowed so that growth arrest of the epiphysial plate occurs. Discrepancies between clinical and experimental results of removal of part of the epiphysial cartilage could be due to one or a combination of factors.

Atypical rachitic changes have been described in man which in some ways resemble those seen in strontium rickets. Here, particularly in long-standing “renal rickets,” nodules of
cartilage have been described (Greene 1922, Parsons 1927, Mitchell 1930, Gilmour 1947). This condition is associated with chronic renal disease and is characteristically intermittent in nature, demonstrated well by multiple "arrest lines" in the metaphysis (Mitchell 1930, Snapper 1943). Here the mechanism of cartilage nodule formation is unknown, although Gilmour (1947) considered that the extreme "osteitis fibrosa" is responsible for the accumulation of cartilage due to interference with endochondral ossification. This assumption is not supported by the present study, for there is no evidence of increased bone resorption and intertrabecular fibrosis. Instead, it is more likely that the intermittent process leads to periodic disturbances of the vascular architecture and sequences of endochondral ossification. Furthermore, McMaster (1935) demonstrated that in healing avitaminosis D rickets small nodules of cartilage remain in the epiphysis and metaphysis.

In addition to the cartilage nodule formation in long-standing rickets, abnormal cartilage masses appear in a number of conditions in man, some considered developmental in origin and others more closely resembling tumours (Fairbank 1951). The mechanism of their development in most cases is unknown, and it is possible that the mechanism of nodule formation seen in strontium rickets could be common to one or more of the conditions such as multiple exostoses, dyschondroplasia and even osteochondritis. For this reason, comparison of experimental and naturally occurring changes is appropriate.

In the condition in which multiple exostoses appear on growing bones, Virchow (1891) considered that bits of cartilage became "snared off" from the lateral margins of the epiphysial plate and, moving on to the body of the bone, initiated the lesion. Keith (1919) thought that for this to happen the perichondral connective tissue must be deficient and in view of the defective growth and remodelling of the bone shaft, he termed the condition diaphysial aclasis. Some degree of failure of longitudinal growth has since been confirmed (Solomon 1961), but no clinical or experimental work has been done to support Keith's (1919) suggestion. Other authors consider that exostoses may arise from periosteal tissue. This arose from observations that trauma and implantation of bladder epithelium may induce cartilage formation from periosteal tissue (Jacobson 1940, Cohen and Lacroix 1955). Jaffe (1943, 1958), after histological study of the well developed lesion, agreed that both perverted perichondral activity and periosteal cartilage formation are compatible pathogenic processes leading to the formation of multiple exostoses. In view of the finding in strontium rickets, a further explanation exists: namely, that local metaphysial vascular and calcification defects are responsible for periodic invagination of the cartilage plate with peripheral sequestration of cartilage nodules on the outer surface of the bone shaft. Although no exostoses formed in the rat during the development of strontium rickets, this is not surprising in view of the short growth period of the animal and the short duration of the experiment. Similarly, solitary endochondromata may occur by the same mechanism for they also have been assumed to be derived from nests of cartilage snared from the epiphysial plate (Geschickter and Copeland 1949).

There is no evidence of inhibition of calcification mechanisms in this condition although recently Todd, Hill, Nickerson and Tingley (1961) have suggested a relationship between hereditary multiple exostoses, pseudo-hypoparathyroidism and other genetic defects of bone with defects in calcium and phosphorus metabolism.

In contrast to the above conditions, multiple endochondromatosis (Ollier's disease, dyschondroplasia, chondrodysplasia) appears different. Although multiple exostoses and endochondromatosis have been considered different manifestations of the same condition, they have been separated on clinical and developmental grounds into distinct entities (Jaffe 1943, Fairbank 1951). In dyschondroplasia, cartilage derived from the epiphysial plate accumulates in the metaphysis, persists for a long time and, when multiple biopsies have been performed, has been described as a benign proliferative lesion of cartilage (Murray and Cruickshank 1960). Few histological studies have been performed and radiographic evidence
does not suggest a nodular deformity of the cartilage plate. However, Speiser (1925) described one case in which cartilage masses were still connected to the epiphysial plate by thin stalks; an observation similar to that sometimes seen in strontium rickets. Furthermore, it is of interest that Müller and Sissons (1951) commented that a case of "metaphysial dysostosis" diagnosed clinically bore a striking resemblance to Speiser's case and yet when examined histologically was shown to be "renal rickets." Here large masses of cartilage were found in the metaphysis as well as pronounced intertrabecular fibrosis and giant cells at bone margins. Later Cameron, Young and Sissons (1954) described a characteristic case of metaphysial dysostosis with little resemblance to renal rickets histologically and considered the condition a separate entity. In another patient an association with rickets was demonstrable radiologically (Blount 1930), but in most cases bone in histological specimens has not been reported abnormal (Hunter and Wiles 1935; Carleton, Elkington, Greenfield and Robb-Smith 1942; Murray and Cruickshank 1960) although study of undecalcified bone sections would be required to confirm normal calcification of trabeculae. For this reason radiological evidence is clearly not sufficient to distinguish between some cases of renal rickets and the chondrodystrophies.

It has been suggested that dyschondroplasia arises from some primary vascular defect, but experiments attempting to show such a pathogenesis are unconvincing (Hunter and Wiles 1935). Furthermore, in Maffucci's syndrome, in which haemangiomata are associated with multiple enchondromatosis, no direct anatomical association has been found between the two lesions (Bean 1955).

From the above evidence it appears that while rickets may occasionally mimic one of the dysplasias of cartilage, the basic change in the case of dyschondroplasia is not similar.

Cartilage nodules also appear in the epiphysis and metaphysis in osteochondritis juvenilis (Haythorn 1949, Ponseti 1956) in which etiological factors must be many (King 1935) and, although rickets was associated with a high proportion of cases before the discovery of vitamin D (Sundt 1921), the pathogenesis of the epiphysial changes is now considered to be one of "avascular necrosis" (Gill 1940, Trueta 1957). It has been pointed out that experiments demonstrating osteochondritis after interruption of blood supply to the epiphysis (Lemoine 1957) does not necessarily mean that this is the etiological factor (Ponseti 1956). Indeed, Ponseti (1956), studying the histology of biopsy material, considered that abnormal changes in the cartilage plate occur first and epiphysial "damage" occurs afterwards. This suggestion is supported by the radiological studies of Gill (1940), showing the cyclic course of the condition, the first manifestation being the appearance of radiolucent areas in the metaphysis. The cartilage plate and epiphysial changes in strontium rickets follow the same developmental course and demonstrate clearly that avascular necrosis is not the only way in which epiphysial deformity can develop. Indeed Ponseti (1956) considered that avascular necrosis might follow epiphysial plate perforation, a suggestion which still requires confirmation.

Thus two mechanisms exist which may lead to "osteochondritis": avascular necrosis and localised defects in endochondral ossification.

**SUMMARY**

1. Stable strontium in large amount in the diet of rats initially inhibits calcification and induces rickets.
2. Changes later become atypical and a complex series of epiphysial plate defects develops: formation of localised osteoid wedges in the metaphysis; invagination of the epiphysial plate and sequestration of multiple cartilage nodules into the marrow cavity; and, in severely affected animals, localised loss of part or parts of the epiphysial plate with formation of large cartilage nodules in the metaphysis and epiphysis.
3. The appearance of cartilage nodules in the metaphysis in man has been shown to be
Experimental epiphysial cartilage growth anomalies

associated with changes in the epiphyseal plate, but much of the information is radiological and therefore incomplete, and detailed cellular changes are seldom available.

4. Some of the conditions mentioned, which have presented difficulty in interpretation, partly because of their rarity but also because of lack of knowledge of the fundamental processes concerned, are multiple exostoses and endochondromatoses, metaphysial dysostosis and osteochondritis.

5. Comparison of basic mechanisms revealed in this study with those supposed to occur in human cartilage dystrophies demonstrates that strontium rickets mimics some changes occurring in chronic renal rickets; that invagination of the epiphyseal plate and cartilage nodule sequestration could account for the development of multiple exostoses and some endochondromatoses; and that localised endochondral defects in calcification can induce epiphysial changes resembling osteochondritis juvenilis, demonstrating that avascular necrosis is not necessarily the mechanism initiating epiphysial deformity.

This work was carried out under a grant from the National Health and Medical Research Council of Australia.

REFERENCES


Bean, W. B. (1955): Dyschondroplasia and Hemangiomata (Maffucci’s Syndrome). Archives of Internal Medicine, 95, 767.


